Biological roles of a species-specific orphan receptor in functional diversity of GnRH signaling via GPCR heterodimerization in the ascidian, *Ciona intestinalis*

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The species-specific paralogous forms of gonadotropin-releasing hormones (GnRHs) and their receptors (GnRHRs), which belong to the Class A GPCR family, have led to the diversity of GnRHergic system during the evolution. Seven GnRH forms, and a GnRHR, Ci-GnRHR1 (R1) and its three paralogs (R2-4) were identified in the protochordate, *Ciona intestinalis*. Unlike other paralogs, R4 fails to bind to any *Ciona* GnRHs and to activate any signaling pathways, suggesting R4 to be an orphan or non-functional receptor. However, R4 heterodimerizes with R1, and the resultant heterodimer elicits 10-fold more potent Ca^{2+} mobilization than R1 in a ligand selective manner, but not modulates cAMP production of R1. Intriguingly, R4 also heterodimerizes with R2, but the R2-R4 decreases the cAMP production in a ligand non-selective manner, compared with R2 alone. These results indicate that R4 is not an inactive receptor, but serves as a protomer of GPCR heterodimers responsible for modulation of the differential signaling of the *Ciona* GnRHRs. Collectively, the present study shows that the GPCR heterodimerization with the species-specific orphan receptor paralog results in the modulation of the multiple GnRHergic signaling, and enables the unique rigorous regulation of the reproductive functions in the protochordate lacking gonadotropins and a pituitary.